Behavioural effects of d-amphetamine in genetically selected lines of rats : prenatal exposure

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BEHAVIOURAL EFFECTS OF d-AMPHETAMINE in GENETICALLY SELECTED LINES of RATS: PRENATAL EXPOSURE*

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Abstract

Active avoidance behaviour of adult rats was assessed following prenatal exposure to d-amphetamine sulfate. Pregnant rats of the RHA/Lu and RLA/Lu genetic lines were administered either 3 mg/kg d-amphetamine sulfate, or a placebo (physiological saline) from gestation Day 6 until gestation Day 20. Differences in avoidance learning of prenatally treated offspring over four days of training, as well as under the effect of three levels of d-amphetamine and a placebo (subsequently administered over four successive days), were analysed according to the complexity of an avoidance response elicited in an either-way avoidance task. Prenatal treatment with d-amphetamine contributed significantly to rates of avoidance response improvement over four days of training. A statistically significant genetic line X prenatal treatment X dose response interaction was observed in one-way ($p < .03$) and two-way ($p < .007$) avoidance under the effect of d-amphetamine. Results were discussed in terms of the inverted-U arousal function, and it is proposed that the prenatal pharmacological intervention may affect baseline levels of physiological arousal. This effect may enhance or impair learning performance, depending upon the complexity of the response and the genetic line.

In the past two decades considerable research interest has been directed toward the investigation of possible harmful effects of drugs administered to the organism in utero. Particular emphasis had been placed upon the physiological anomalies found in offspring with a prenatal history of pharmacological intervention. This research approach has assumed other directions. There has been a growing concern that in determining a drug's teratogenic potential more data need be considered than mere reliance upon morphological indices. Increasing awareness of subtle teratogenic effects without obvious morphological changes continues to stimulate research. Intuitively, it would seem reasonable to continue to suspect a drug's possible teratogenic action, in spite of the consideration that no physical defects may be noticed post partum. Indeed, there is evidence that teratogenic effects are not necessarily limited to anatomical and physiological systems. For example, the administration of methyl-mercury in rats during pregnancy can produce enduring behavioural deficits without any observable morphological changes (Coyle, Wayner, and Singer, 1976).

One drug in particular -- amphetamine -- has not been thoroughly investigated in regard to its potentially teratogenic effect on postnatal adult behaviour. Although the amphetamines have been studied extensively, it is to be recognized that based upon the diversity of

research strategies employed, and the range of behavioural data which have been reported, a more definitive understanding of its teratogenic action has yet to be achieved.

Behavioural teratology. Teratology is the study of abnormalities arising during prenatal development (Clegg, 1971). For many years studies have been restricted mainly to the investigation of morphological abnormalities (Friedler and Cochin, 1972; Hook, 1971; Nora, Trasler, and Fraser, 1965; Petit and Sterling, 1977). It has become evident that the behavioural and functional adaptation of the offspring to its environment may also be susceptible to teratogenic effects of drugs. Behavioural change may be a more sensitive indicator of a drug's suspected teratogenic action (Golub and Kornetsky, 1974). If the developing fetus can be affected by stressor agents, it is not unreasonable to suppose that some of these agents might influence the behavioural development and subsequent adjustment of the organism (Seliger, 1973). Possibly, pharmacologically induced changes in levels of neurotransmitters at critical stages of development could alter subsequent levels of brain neurotransmitters or response thresholds. The altered level of neurotransmitters or response thresholds might then produce changes in the brain which might in turn result in behavioural changes (Middaugh, Blackwell, Santos, and Zemp, 1974).

In mammals the teratogenic effects of any drug are determined primarily by the following factors: chemical

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nature of the teratogenic action; stage of development of the organism at the time of drug administration; and the susceptibility of the species and individual sensitivity (Wilson, 1968). Drug induced behavioural deficits can result from deficiencies of precursors, substrates, enzymes and other substances necessary for normal cell metabolism; enzyme inhibition; osmotic imbalance; and changes in cell membrane characteristics (Wilson, 1973). It seems reasonable to assume that drugs which affect the central nervous system (CNS) are more likely to cause congenital behavioural anomalies (Coyle, Wayner, and Singer, 1976). Although any interferences with prenatal life can be considered potentially teratogenic (Joffe, 1969; Wilson, 1968), the embryo is generally resistant prior to implantation and after organogenesis (gestation days in the rat of 1 - 6, and 17 - 22, respectively). In mammals the CNS has the longest period of development. The most vulnerable period with respect to behavioural dysfunctions has not been determined (Coyle, Wayner, and Singer, 1976).

It has been demonstrated that the placenta does not act as a filter to protect the developing organism from harmful agents in the maternal blood stream (Villee, 1965; Yaffe, 1966). The results of some studies (Hammond and Toseland, 1970; Shearer, Schreiner, and Marshall, 1972) have indicated that psychoactive drugs may pass through the placenta without delay. The main protection afforded by the

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placenta is to slow the transfer of substances thereby providing more time for the maternal organism to metabolize and excrete the substance before the concentration in the embryo or fetus becomes injurious (Coyle, Wayner, and Singer, 1976).

Amphetamine. Amphetamine is a central nervous system stimulant drug with sympathomimetic properties, and a catecholamine-releasing action. d-Amphetamine is one of the most potent sympathomimetic amines. It owes its behaviourally facilitative action to norepinephrine (NE) release (Stein and Wise, 1970). Amphetamine promotes the release of catecholamines from peripheral and central adrenergic nerve terminals (Groves and Rebec, 1976). At higher doses than those required to demonstrate an in vivo catecholamine release, amphetamine gains the ability to block the uptake of exogenous catecholamine across the neuronal membrane (Fuxe and Ungerstedt, 1968). At concentrations of the drug still higher than those required to enhance release or block the uptake of catecholamines, amphetamine may also inhibit monoamine oxidase activity (Glowinski, Axelrod, and Iverson, 1966). It is known that chronic amphetamine administration in the adult rat results in a significant depletion of brain catecholamines. Norepinephrine levels are especially reduced among nerve terminals in the reticular formation, hypothalamus, and parts of the hippocampus; while telencephalic dopamine (DA) levels are also diminished (Ellinwood and Escalante,

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1970).

In animals, amphetamine has the effect of producing an individual with heightened sensitivity to external stimuli. Reviews by Cole (1967, 1970) present evidence for the facilitating effect of amphetamine on intellectual and motor performance, bodily activity, peripheral systems, avoidance conditioning, and escape behaviour. Cole (1970) reports that d-amphetamine may facilitate the monitoring of cues without actually increasing the response time, thus producing increased alertness or improved discrimination.

Amphetamine administered during gestation. Offspring of rats injected with 3 mg/kg amphetamine (gestation days 12 - 15) had depressed activity levels at 45 days of age in an open field test (Bell, Drucker, and Woodruff, 1965). Results were attributed to increased emotionality of offspring, as the animals were exposed to immobilization stress before testing. In contrast to these findings, Clark, Gorman, and Vernadakis (1970) found no effect of prenatal amphetamine (1 mg/kg, gestation days 12 - 15) on open-field activity in offspring at 13, 15, 18, 46, or 60 days of age. There was no effect on learning or reversal in a T-maze, nor was there an effect on performance or extinction of an operant response. Prenatal amphetamine offspring did show a significantly lower problem solution latency in a mother-goal maze. These offspring were tested before 35 days of age. Seliger (1973) injected gravid rats with 5.0, or 10.0 mg/kg d-amphetamine during gestation days 5 - 9, or

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12 - 16. Administration of 5 mg/kg d-amphetamine to mothers early in pregnancy was detrimental to offspring's learning of a passive avoidance response. Interpretation of this finding may be confounded by the consideration that at the time of drug administration, there were no catecholamines in the brain (Mabry and Campbell, 1977). Middaugh et al., (1974) report d-amphetamine (5 mg/kg) administered to mice during the third trimester produced transient alterations in catecholamine concentrations, and increased open-field activity levels in 75 day-old offspring. Nasello, Astrada, and Ramirez (1974) injected gravid rats with 0.5 mg/kg d,l-amphetamine throughout gestation. This treatment increased the offspring's ability to learn a conditioned avoidance response (in a shuttlebox), and their susceptibility to seizure discharges. Martin (1975), administered 1.0, 3.0, or 5.0 mg/kg methamphetamine HCL twice daily throughout gestation to rats. The 5.0 mg/kg offspring (tested at 100 - 120 days of age) made more conditioned shuttlebox avoidances than did the 3.0 mg/kg methamphetamine prenatally treated offspring and saline prenatally treated offspring. Martin, Martin, Radow, and Sigman (1976) followed the same prenatal administration procedures as Martin (1975), but continued to inject the nursing dams. At 3-months of age animals receiving methamphetamine early in development had higher activity levels (activity wheel-running) than saline treated offspring, which continued for the next seven months. Hitzemann, Hitzemann, Brase, and Loh (1976) injected

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gravid rats with 1.0, or 3.0 mg/kg d-amphetamine from day 5 of gestation to parturition. Eighty-five day-old prenatal amphetamine offspring had significantly higher open-field activity levels and accompanying decreases of diencephalon and brainstem NE, and decreases of brainstem DA, compared to saline controls. Nasello and Ramirez (1978) followed the same prenatal intervention procedures as conducted by Nasello et al., (1974) and reported that prenatal amphetamine offspring had significantly more conditioned shuttlebox avoidances than saline control animals. More recently, Monder (1979) treated gravid rats with either 2.0, 5.0, mg/kg d-amphetamine, or a placebo, mixed with their drinking water, throughout gestation. Offspring injected with amphetamine at 100 days of age demonstrated reduced open-field activity compared to controls, and fewer stand-ups compared to controls. Results of this particular study may be methodologically criticized, as Cole (1977) has reported that differences in size of arenas may significantly alter the effects of d-amphetamine injection on ambulatory activity in adult rats.

As may be seen, there is a considerable body of literature reporting effects of prenatal amphetamine on postnatal behaviour. A variety of methodologies and behavioural measures have yielded data covering a range of developmental and behavioural indices. For example, differing times of pharmacological prenatal intervention, ages of offspring when postnatal effect of prenatal amphetamine administration was evaluated, and techniques employed to assess behaviour

have contributed to a wealth of data. Experimental conditions, such as dosage levels of amphetamine, and possibly the strain of experimental animals may have played a role in determining effect of prenatal intervention on postnatal behaviour. The role of genetic selection has not been systematically investigated in this regard. As well, the problems of distinguishing between transient and permanent effects of behavioural teratogens must be considered. Behavioural deficits which persist into adulthood appear to be the most serious. Perhaps, a more sensitive indication of the teratogenic effects of long-term exposure to a suspected teratogen might be attained through use of active avoidance learning measures. An active avoidance paradigm holds advantage over other developmental or behavioural indices because active avoidance responses may contain differing levels of learning complexity. Therefore, a better opportunity to assess the range of behavioural teratogenic effects of prenatal amphetamine in adulthood could be achieved.

Psychopharmacogenetics. The general topic of psychopharmacogenetics may be understood to cover the whole range of reactions to drugs as modified by genetic determinants (Broadhurst, 1977). More specifically, Eleftheriou (1975) defined it as "the area... which deals with pharmacologic agents that alter a given behaviour in controlled genetic systems" (p 1). In addition, as proposed by Remington and Anisman (1976), the genetic approach also serves as a use-

ful method in delineating the behavioural correlates of drug-induced arousal. Hence, the alteration of a given phenotype in controlled systems by means of prenatal pharmacological intervention may provide data on the genetic bases of behaviour.

Species and individual sensitivity to teratogens varies considerably (Clegg, 1971). Although genetic factors are primarily responsible for determining embryonic reactivity to environmental stimuli, genetic and environmental interactions are complex, and the maternal genotype can also influence reactions of the developing organisms to teratogens (Dagg,1963). Individual differences in drug absorption and metabolism in the maternal organism can determine, in part, embryonic reactivity (Coyle, Wayner, and Singer, 1976). Pharmacological alteration of the prenatal environment may establish a basis for genetic line-specific behavioural teratogenic susceptibility. No studies, to this author's knowledge, have been reported which investigate the effects of chronic amphetamine administration during gestation on the offspring's adult active avoidance behaviour in genetically selected lines of rats. To conduct such a study would seem most appropriate in helping to determine aspects of the genetic-prenatal environment interaction on subsequent behaviour.

Bignami (Bignami and Bovet, 1965; Bignami, 1965) founded the bidirectionally selected lines of rats, now comprising the Roman high-avoidance line (RHA), and the low-avoidance line (RLA). Satinder (1977, 1981 - in press)

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has proposed that these two lines of animals, genetically selected for performance differences on task-specific avoidance learning, have genetically related differences in levels of arousal.

The purpose of the present study was to investigate the effect of chronic prenatal d-amphetamine administration on adult active avoidance learning. d-Amphetamine is a psychoactive drug with proven arousal properties, and research with the drug using animals of the Roman lines has provided insights into the causes of their consistent and reliable differences in avoidance performance (Satinder, 1977). Hence, research into the effect of chronic amphetamine on maturing brain systems was warranted. The present study attempted to evaluate genetic variations in avoidance learning between prenatally treated lines of rats known to exhibit pronounced differences in behaviour as adults. By endeavouring to determine the developmental response to amphetamine via adult performance on active avoidance, the interactive effect of the genotype-prenatal environment relationship may be better understood.

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Method

Part I: Prenatal Pharmacological Intervention

Subjects. Thirty-four experimentally naive, primiparous and multiparous female rats from the RHA and RLA genetic lines were used as subjects. The number of animals used from each genetic line and for each drug treatment are presented in Table 1. To differentiate the lines at Lakehead University from the lines at other places, these have been redesignated as RHA/Lu and RLA/Lu (Satinder, 1971). The laboratory temperature was maintained at 22 ± 1°C. Humidity level was maintained at 40%, and fluorescent lighting was on a 12:12 light/dark cycle. All procedures were carried out in the light phase. A detailed description of the care and maintenance of the animals has been previously given by Satinder and Hill (1974, Experiment 2).

Experimental design. Drug administration via the subcutaneous route was selected because the alternative routes precluded their use. Intravenous administration delivers the compound to its site of action too quickly to afford sustained effect. Oral administration, capable of yielding prolonged effect, involved passage of a stomach tube to ensure proper dosage per animal. This procedure is often quite stressful to the animal. There is danger of possible damage to the trachea and esophagus. Intraperitoneal (ip) administration has obvious hazards when

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applied during pregnancy; inadvertant uterine puncture is quite possible (Seliger, 1973).

Administration of the drug to the females was scheduled to begin on Day 6 of gestation. In the rat implantation occurs 6 days after fertilization (Supplement to Teratology Workshop Manual, 1965). Prior to implantation the rat is relatively refractory to teratogenic treatment, as manipulation during this period may result either in failure to implant, spontaneous abortion, or embryonic death (Wilson, 1974).

Pregnant animals received subcutaneous (sc) injections (at the back of the neck) of either 3 mg/kg d-amphetamine sulfate in a 1 ml physiological saline solution, or 1 mg/kg physiological saline (placebo control), commencing Day 6 of gestation. Subjects were injected twice daily: between 0:800 hrs. - 1000 hrs., and 1600 - 1800 hrs. Dosages were administered so that each subject received the first third of its total daily dosage in the morning and the remaining two-thirds in the afternoon. To maintain the drug's peak effects daily dosages were employed for practical reasons, with one-third and two-thirds dosages corresponding to eight hr. and sixteen hr. injection intervals. Twice daily injections were to ensure that a reasonably constant level of the drug was present in the organism, on a 24-hour basis (Martin, 1975). Dosages were adjusted daily to allow for maternal weight gain. The twice daily injections also reduced the chances for embryonic or early fetal toxicity that a single injection per day may have incurred. Injection

of the dams terminated on Day 20 of gestation. The complications and hazards of parturition while the subjects were under the effect of the drug were thereby averted.

Procedure. The female rats were paired with a male of the same genetic line in individual cages on racks. The morning that a sperm plug was found counted as the beginning of Day 1 of gestation; males were then removed. On Day 6 of gestation the pregnancy was confirmed if the animal's weight that day was minimum 10 grams greater than its pre-pairing weight; females were transferred to individual breeding cages. Animals were allowed ad libitum access to food, and were given a 10% sucrose drinking solution to counteract the possible anorectic effects of d-amphetamine. Animals receiving saline injections also received the sucrose solution. Females were individually coded so the experimenter did not know the genetic origin of the pregnant animal.

At birth the litters were weighed, and a mean pup weight per litter was calculated and recorded. The number of pups per litter was noted as well. Beginning postnatal day (PND) 6 water bottles with 10% sucrose drinking solution were replaced by those containing water.

Pups were weighed, sexed, weaned, re-coded, and placed in same-sex pairs, with the genetic lines on separate cage racks on PND 28. Litters were not culled to the same size in part due to the consideration that little guarantee existed for subsequent survival of remaining pups; and also to provide a larger sample of behavioural effects.

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Part II: Behavioural Testing Of The Prenatally Treated Offspring

Subjects. One hundred twenty-eight offspring of females injected with either amphetamine or saline during gestation from the RHA/Lu and RLA/Lu genetic lines were used as subjects. The number of animals of each genetic line and prenatal treatment which were tested are presented in Table 2. During behavioural testing these animals were coded and housed individually to ensure that the experimenter did not know their genetic origin or prenatal history. Environmental controls were the same as previously mentioned. The ages of the animals at the onset of testing ranged from 65 - 75 days (M = 72 days).

Experimental design. Either-way avoidance learning was selected as a behavioural measure because of its usefulness in delineating avoidance reactions to different levels of response complexity. Either-way performance (responses made as either-way, whereby the animal can run in either direction) may be analysed as having two subcomponents: responses made as two-way (animal returns to the compartment previously occupied); and responses made as one-way (animal runs away from compartment previously occupied). Two-and one-way avoidance tasks, which involve back and forth shuttling responses and responses in one direction, respectively, have been reliably shown to represent different levels of task complexity (Anisman, 1973; Ashe and McCain, 1972; Satinder, 1977, Theios and Dunaway, 1964). An either-way task is less complex than a one-way task (Anisman and Wahlsten, 1974; Satinder, 1977). Hence, either-way avoidance may be examined as containing

three levels of complexity of response; responses made as two-way (greatest complexity), responses made as one-way (intermediate complexity), and responses made as either-way (least complexity).

All animals were tested for the unconditioned escape response (UER) 48 hours before avoidance training because UERs of these genetic lines are known to differ and this difference affects two-way active avoidance (Satinder, 1976).

During training and under the effect of d-amphetamine an avoidance trial included a maximum of 10 seconds of conditioned stimulus (CS) alone, followed by 10 seconds of both CS and unconditioned stimulus (US) and a 40-second intertrial interval (ITI). The CS was a 70-dB 9-kHz pure tone against a background noise of 40 dB. The speaker was located in the centre of the circular runway. Sound intensity was measured at floor level above the standard reference level of 0.0002 μbar by a General Radio sound level meter, Type 1551-C. The shock intensity equivalent to the UER of each animal was used as the US level in avoidance learning. The CS was followed by the US if no avoidance response occured within the CS duration. The CS terminated immediately after an avoidance response, and both CS and US terminated immediately after an escape response. The first training trial was an escape trial for each animal (Satinder, 1977).

Testing of the animals was scheduled to begin on PND 70. For practical reasons all offspring could not be tested at exactly the same age. Technical limitations,

such as numbers of animals to be tested concurrently in the single apparatus on a daily basis, required that this date be modified. Offspring were assigned to groups; each group of approximately equal age was successively tested.

Apparatus. The apparatus consisted of a circular Plexiglas runway (12 cm wide and 15 cm high, with an outside circumference of 220 cm) which could be divided into four equal compartments by guillotine doors. The runway floor was constructed of 0.25 cm stainless steel rods spaced 1 cm apart (centre to centre). The apparatus delivered a scrambled electric shock to the grids. The shock unit contained a primary 115-V ac power source and a step-up transformer with a secondary rating of 3000V (Hammond Model 216-60). The shock was delivered through a 2.7 X 10^6 ohms resister. A digital clock was used to record response latencies (Satinder, 1977).

Procedure. In determining each animal's UER, the animal was individually adapted in the circular runway for a 1-minute period prior to receiving foot shock. Electric shock was administered until the animal escaped within 5 seconds, running at least a distance equivalent to a quarter length of the runway (all doors open), in either direction. This constituted a UER. Each animal was given 10 trials of the ascending series by using the method of limits, with an ITI of approximately 5 seconds. Shock intensities ranged between .27 - .97 mA in 22 steps. The lowest electric

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shock intensity to elicit UER reliably for each animal was determined in this manner (Satinder, 1976).

In either-way avoidance the first trial on each day was identical to that of one-way response (Satinder, 1977). On subsequent trials both doors of the compartment occupied by the animal were raised, which permitted the animal to return to the compartment previously occupied or to run away from the compartment. The height of the door opening was adjusted according to the size of each animal such that the rat had to squeeze under the door. This was done to make the "crossing response" very distinctive to the rat. The direction taken by the animal (into the previously occupied compartment, or away from it) for each trial was recorded (Satinder, 1977).

Every animal was given four training sessions of 10 trials each on four successive days (Satinder, 1977).

Three levels of d-amphetamine sulfate (1.0, 2.0, and 4.0 mg/kg) and a placebo (physiological saline) were administered to each animal on four successive days (Satinder, 1977). The two lowest levels of the drug employed in the testing corresponded to the morning and late afternoon levels, respectively, administered to the offspring in utero. The drug was administered ip in physiological saline in a volume of 2 ml/kg, approximately at the same time of day, 30 minutes before testing. The order of dosages was assigned at random, and then within each dosage an approximately equal number of animals started their drug schedule

and rotated through the remaining dosages in the order assigned (Satinder, 1977).

The procedure for testing during the effects of the drug was the same as during training. A double-blind technique was used. The person recording the responses did not know the genetic line, history of prenatal treatment, or the drug dosage.

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Results and Discussion

Reproductive and behavioural data were analysed according to prenatal treatment, i.e., d-amphetamine and saline treated animals, and according to genetic line. Differences with associated probabilities less than .01 were considered significant.

Part I: Prenatal Pharmacological Intervention

The means and frequencies of reproductive data are presented in Table 1. Data were based on the means for the litters weaned. Results were evaluated by t-test, or chi-square, where appropriate. No differences were found between prenatal treatment groups nor between genetic lines in any aspect of data.

Satinder (1980) has examined the reproductive behaviour of these genetically selected lines of rats, and found no differences between the lines. The administration of 1.0, 3.0, mg/kg d-amphetamine daily to gravid female rats from the fifth day of gestation until parturition showed no differences in size or weight of the litters between saline-and amphetamine-treated groups (Hitzemann, et al., 1976). The present findings are consistent with these findings.

Two pups from two different RLA dams which received prenatal d-amphetamine did exhibit morphological anomalies

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Table 1

Means and Frequencies of Reproductive Data

Genetic lines	RHA/Lu		RLA/Lu	
Prenatal Treatment	Amphetamine	Saline	Amphetamine	Saline
Total Number of Pairings	12	7	9	6
Total Number of Litters	8	7	8	4
Number of non-deliveries	4	0	1	2
Number of Dams Destroying Litters	2	3	2	0
Total Number of Litters Weaned	6	4	6	4
Number of Gestation Days	22.1	23.1	22.9	22.8
Number of Pups At Birth	6.2	9.5	8.7	8.3

(continued)

Table 1 (continued)

Genetic lines	RHA/Lu		RLA/Lu	
Prenatal Treatment	Amphetamine	Saline	Amphetamine	Saline
Number of Pups Weaned	5.2	7.8	7.2	6.5
Total Number of Offspring	31	31	41	25
Pup Weight At Birth (g)	5.5	5.4	5.8	6.0
Pup Weight At Weaning (g)	48	51	53	50
Number of Females Weaned	3.0	4.8	2.8	2.8
Number of Males Weaned	2.2	3.0	4.3	3.8
Body Wgt. of Females at Weaning (g)	48	50	48	47
Body Wgt. of Males at Weaning (g)	50	52	55	51

at birth. One animal was hydrocephalic, living until 34 days of age. The other was born without eyes, and survived to maturity; but was not included in behavioural testing. Neither of these abnormalities have been observed in this laboratory, nor have they been reported in the previous literature, for the dosage level used in this study.

Part II: Behavioural Testing Of The Offspring

An analysis of litter differences within each of the genetic line - prenatal groups did not indicate any significant effects; hence litter effect was not included as a variable in subsequent analyses.

The distribution of subjects per experimental group is presented in Table 2. Due to limited computer facilities repeated measures analysis of variance could be performed only with an equal number of subjects per cell. As the smallest number of subjects in one group was 10 (RLA saline prenatally treated females, refer to Table 2) a 2(genetic line - GL) X 2(sex) X 2(prenatal treatment - PNT) repeated measures analysis of variance with 80 animals (n = 10 subjects/cell) was used. The number of subjects for each of the remaining (seven) experimental groups was reduced to 10 through random selection.

In general, there were no significant differences between sexes. For this reason the sex variable was deleted and data pooled for further analysis. Deletion of the sex variable permitted the inclusion of 25 animals per cell in a 2(GL) X 2(PNT) analysis. Numbers of subjects for each of

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Table 2

Distribution of Prenatal Offspring
Per Experimental Group

	RHA		RLA	
	Amphetamine	Saline	Amphetamine	Saline
Female	18	19	16	10
Male	13	12	25	15
Total	31	31	41	25
No. of litters	6	4	6	4

the RHA saline-amphetamine prenatal treatments, as well as the RLA amphetamine prenatal treatment were reduced to 25 through random selection, regardless of sex. Comparison of the analyses involving 128 (the number of animals tested) and 100 subjects each did not show any obvious differences in any of the three aspects of behaviour (i.e., either-way, one-way, two-way avoidance). Indeed, avoidance rates representing the most complex level of response (two-way avoidance) derived from each analysis were quite similar; and for this reason are presented in Figure 1. Hence, subsequent repeated analyses of variance on avoidance behaviour during training as well as under the effect of d-amphetamine were conducted with 100 subjects (n = 25/cell).

As mentioned previously, avoidance performance in the either-way task could be analysed according to the complexity of the response: Each time an avoidance was recorded, the direction taken by the animal in the apparatus was noted. This yielded data on the number of either-way avoidances, comprised of one-and two-way responses, made by each animal during training as well as under the effect of d-amphetamine. Mean numbers of responses per experimental group for each day of training, and under each of three levels of d-amphetamine and the placebo were evaluated by analyses of variance; and are presented in Figures 2, 3, and 4 for either-way, one-way, and two-way responses, respectively.

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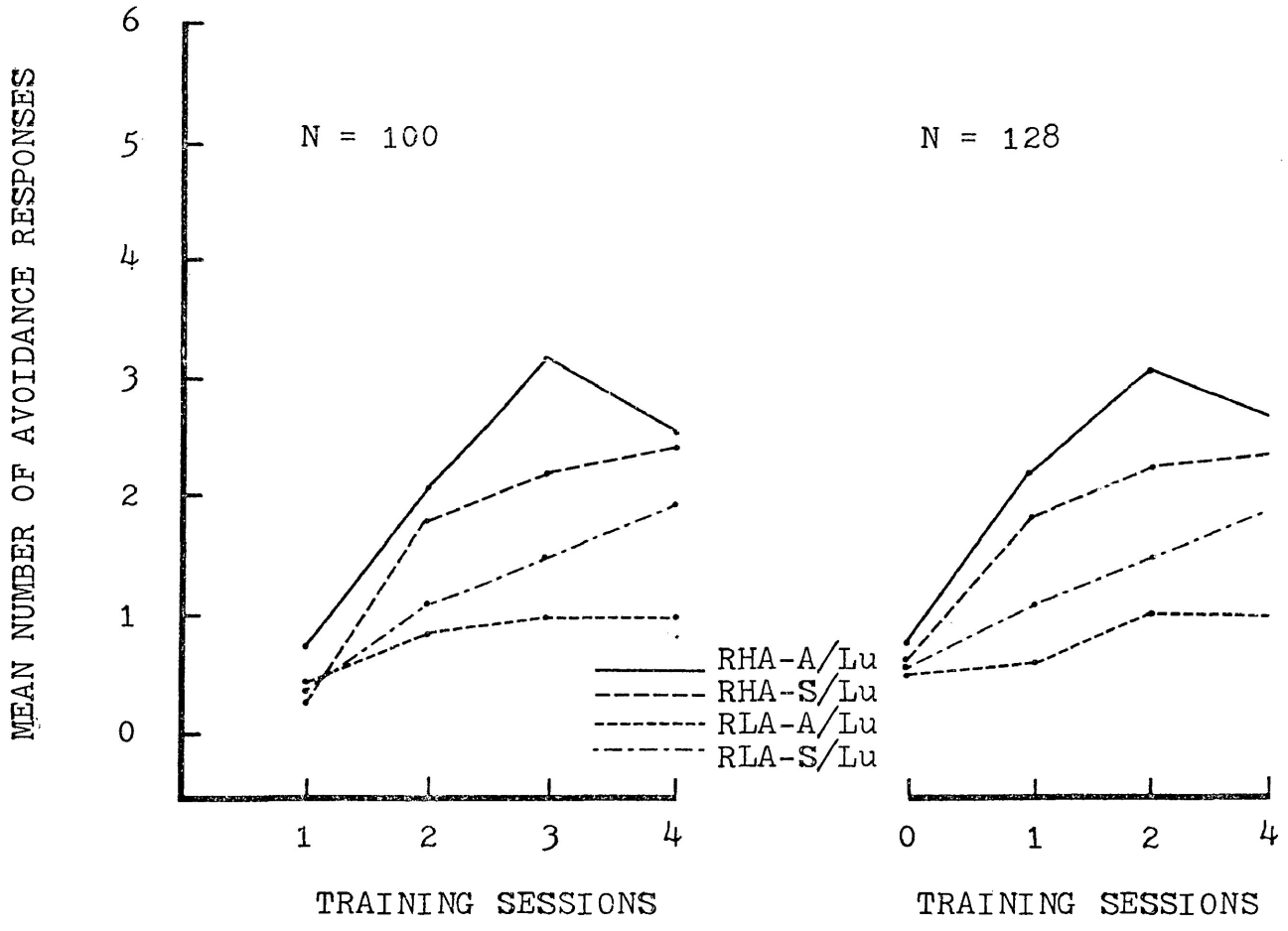

Figure 1. Mean number of two-way avoidance responses of two lines of rats and prenatal treatments with an n = 100 and n = 128.

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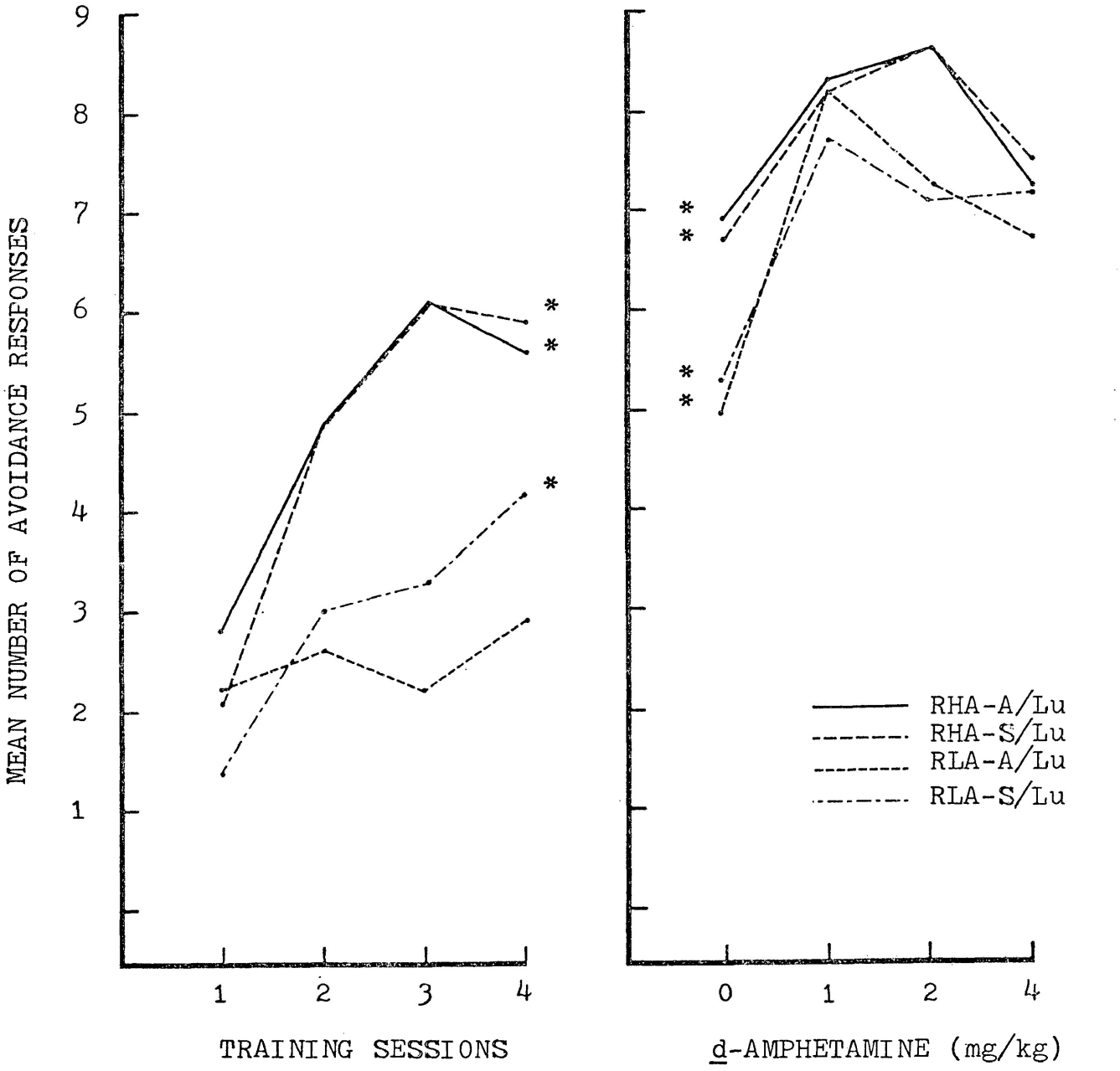

Figure 2. Mean number of either-way avoidance responses of two lines of rats (each of two different prenatal treatments) during training and under the effect of d-amphetamine, in an either-way avoidance task. (Response curves showing significant changes are marked with an asterisk.)

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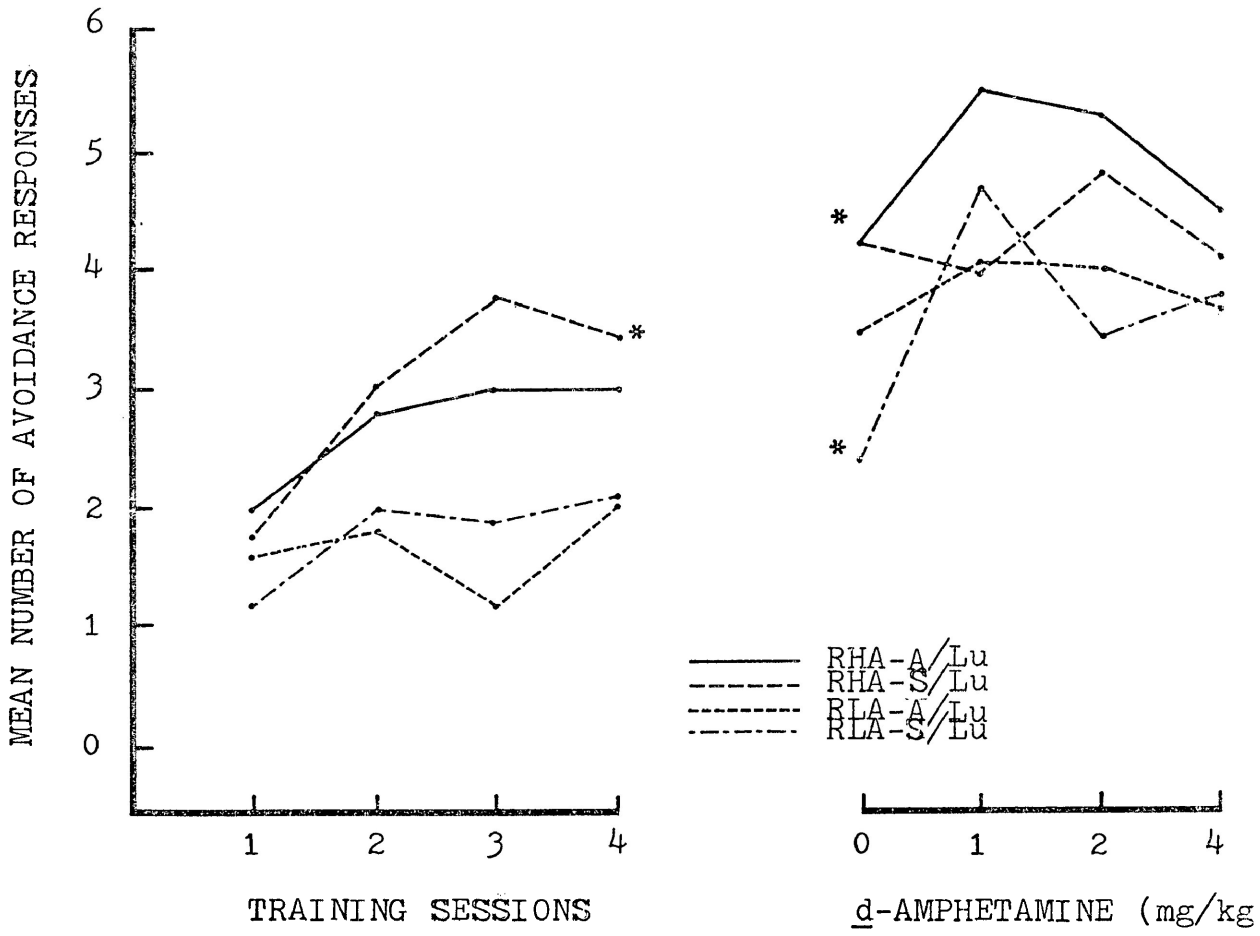

Figure 3. Mean number of one-way avoidance responses of of two lines of rats (each of two different prenatal treatments) during training and under the effect of d-amphetamine, in an either-way avoidance task. (Response curves showing significant changes are marked with an asterisk.)

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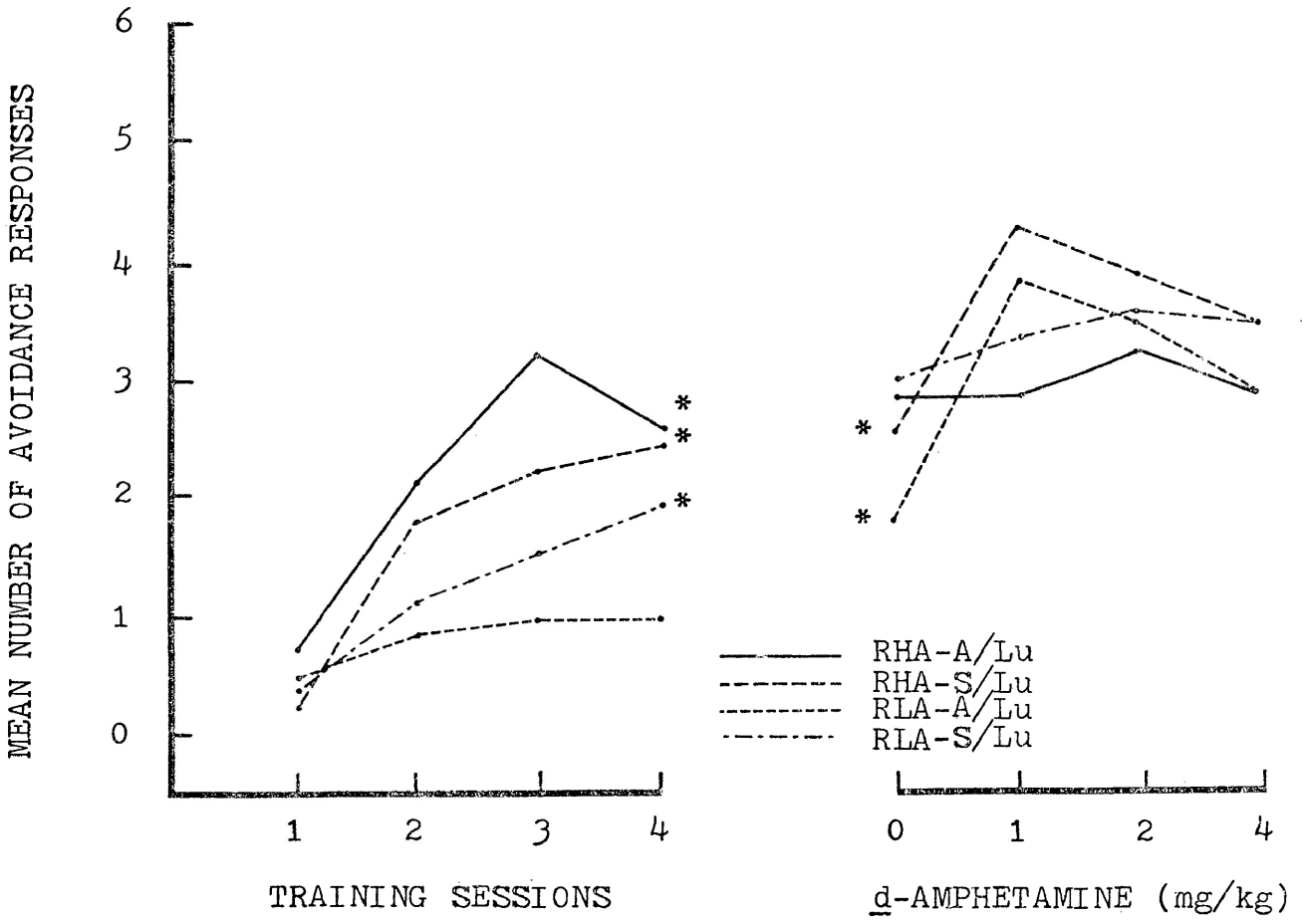

Figure 4. Mean number of two-way avoidance responses of two lines of rats (each of two different prenatal treatments) during training and under the effect of d-amphetamine, in an either-way avoidance task. (Response curves showing significant changes are marked with an asterisk.)

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Training. During training prenatal d-amphetamine animals, irrespective of genetic line, made higher numbers of either-way (Figure 2), and one-way (Figure 3) responses, and lower numbers of two-way (Figure 4) responses, than the prenatal saline treated animals. However, none of these differences were statistically significant. As indicated in Figures 2, 3, and 4, respectively, animals of the RHA genetic line showed higher response rates during training than animals of the RLA genetic line in all the three aspects of behaviour, i.e., either-way avoidance ($p < .001$); one-way avoidance ($p < .001$); and two-way avoidance ($p < .001$ -- refer to Table 3 for statistical presentation of differences between genetic lines and prenatal treatment during training). Satinder, (1977) using similar testing procedures found RHA animals had significantly higher response rates than RLA animals in the either-way task during training: By comparing numbers of one-and two-way avoidances made by each genetic line in the either-way task during training, RHA animals responded at higher levels than RLA animals in both aspects of behaviour, i.e., one-and two-way avoidance (Satinder, 1977). Hence, present findings are in agreement with previous findings. In general, significant improvement in performance level over four days of training was noted in all the three aspects of behaviour, i.e., either-way avoidance ($p < .001$); one-way avoidance ($p < .001$); and two-way avoidance ($p < .001$, refer to Table 3). However, as indicated in Table 3, animals

Table 3

F-ratio and Levels of p Value for the Differences Between Genetic Lines and Prenatal Treatments During Training

	Either-way	One-way	Two-way
Genetic lines - GL - (df 1,98)	27.5***	19.5***	14.8***
Prenatal treatment - PNT - (df 1,98)	0.2	0.9	0.01
GL - saline PNT (df 1,48)	9.2**	9.5**	2.0
GL - amphetamine PNT (df 1,48)	20.4***	10.5**	16.9***
GL X PNT (df 1,96)	0.5	0.2	3.5
Training effect over 4 days (df 3,288)	39.1***	9.6***	30.9***
training effect X GL (df 3,288)	9.1***	3.7*	5.2**
training effect X PNT (df 3,288)	3.0*	2.4	1.1
training effect X GL X PNT (df 3,288)	0.6	0.1	1.1
training effect - saline PNT (df 3,144)	27.2***	8.7***	19.7***
training effect - amphet PNT (df 3,144)	13.9***	2.7	13.2***
training effect - amphet PNT (df 3,72)	RHA:22.7***	RHA:3.2*	RHA:16.2***
	RLA:1.9	RLA:1.8	RLA:1.0
training effect - saline PNT (df 3,72)	RHA:22.7***	RHA:8.1***	RHA:12.9***
	RLA:14.0***	RLA:1.9	RLA:7.5***
training effect X GL X amphet PNT (df 3,144)	9.5***	2.5	5.2**
training effect X GL X saline PNT (df 3,144)	2.3	1.8	1.1

(continued)

Table 3 (continued)

	Either-way	One-way	Two-way
training effect within GL X PNT (df 3,144)	RHA-A x RHA-S: 0.6	RHA-A x RHA-S: 1.2	RHA-A x RHA-S: 0.7
	RLA-A x RLA-S: 2.9*	RLA-A x RLA-S: 1.2	RLA-A x RLA-S: 1.6

*p < .05
**p < .01
***p < .001

receiving prenatal d-amphetamine did not demonstrate significant learning during training in one-way (Figure 3) avoidance.

The interaction between training and d-amphetamine prenatally treated genetic lines was significant in either-way avoidance ($p < .001$, Table 3), and in two-way avoidance ($p < .002$, Table 3). No corresponding interactions were statistically significant in any aspect of avoidance behaviour in the performance of saline prenatally treated genetic lines. Hence, prenatal treatment with d-amphetamine contributed significantly more to the differences in learning between genetic lines in two-way (Figure 4) and either-way (Figure 2) avoidance.

In either-way avoidance animals of the RHA genetic line receiving prenatal d-amphetamine (RHA-A), and prenatal saline (RHA-S) both demonstrated significant learning during training (Figure 2, $p < .001$, Table 3). The RLA-S animals demonstrated significant improvement during training ($p < .001$), and the RLA-A animals did not; and the resultant interaction was marginally significant ($p < .04$, Figure 2, Table 3).

In one-way avoidance (Figure 3) only the RHA-S animals showed significant learning during training ($p < .001$, Table 3).

In two-way performance (Figure 4) all groups except RLA-A showed improvement during training ($p < .001$, refer to

Table 3).

During training RHA-S animals had higher response rates than RLA-S animals ~~(refer to Table 3)~~. However, these differences were significant in either-way and one-way avoidance ($p < .004$); but were not in two-way avoidance. RHA-A animals had higher response rates than RLA-A animals in all the three aspects of behaviour during training, i.e., either-way ($p < .001$); one-way ($p < .003$); and two-way ($p < .001$) avoidance. During training prenatal treatment with d-amphetamine contributed at a higher magnitude to the differences between genetic lines in two-way (more complex) avoidance which were not evident between saline prenatally treated genetic lines.

Effect of d-amphetamine. In general, animals of the RHA genetic line demonstrated higher response rates under the effect of d-amphetamine in all the three aspects of behaviour. As indicated in Table 4, these differences were significant only in one-way avoidance (Figure 3, $p < .006$). Satinder (1977) reports that genetic line differences in either-way avoidance under the effect of d-amphetamine were not significant. Hence, present findings are in partial agreement with previous findings. Prenatal treatment had no significant effect on any of the three aspects of response complexity under the effects of the three levels of d-amphetamine and a placebo (see Table 4).

Significant dose response (Table 4) was found in all the three aspects of avoidance behaviour, i.e., either-way

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Table 4

F-ratio and Levels of p Value for the Differences Between Genetic Lines and Prenatal Treatments Under the Effect of d-Amphetamine

	Either-way	One-way	Two-way
Genetic lines - GL - (df 1,98)	4.3*	7.9**	0.1
Prenatal treatment - PNT - (df 1,98)	0.0	1.9	2.7
GL - saline PNT (df 1,48)	2.0	3.3	0.2
GL - amphet PNT (df 1,48)	2.2	3.9	0.1
GL X PNT (df 1, 96)	0.0	0.3	0.1
Dose response (0 x 1 x 2 x 4mg/kg) (df 3,288)	22.5***	6.0***	7.6***
dose response X GL (df 3,288)	2.7*	1.5	0.1
dose response X PNT (df 3,288)	0.3	0.2	0.2
dose response X GL X PNT (df 3,288)	0.2	3.1*	4.1**
dose response - saline PNT (df 3,144)	9.1***	2.9*	3.3*
dose response - amphet PNT (df 3,144)	14.1***	2.8*	4.8**
dose response - amphet PNT (df 3,72)	RHA: 6.5***	RHA: 2.5	RHA: 0.9
	RLA: 8.2***	RLA: 0.6	RLA: 6.9***
dose response - saline PNT (df 3,72)	RHA: 6.5***	RHA: 0.8	RHA:4.4**
	RLA: 8.1***	RLA: 5.8**	RLA: 0.4
dose response X GL X amphet PNT (df 3,144)	2.2	0.3	2.7*
dose response X GL X saline PNT (df 3,144)	1.1	3.7*	1.4

(continued)

Table 4 (continued)

	Either-way	One-way	Two-way
Dose response (0 x 1mg/kg) - amphet PNT (df 3,144)	no differences	RHA: 6.26*	RHA: 0.01
		RLA: 1.3	RLA: 25.4***
Dose response (0 x 1mg/kg) - saline PNT (df 3,144)		RHA: 0.05	RHA: 14.9***
		RLA: 25.4***	RLA: 0.6
dose response (0 x 1mg/kg) X GL - amphet PNT		0.72	10.4**
dose response (0 x 1mg/kg) X GL - saline PNT		12.5***	5.3*

*p < .05
**p < .01
***p < .001

avoidance ($p < .001$); one-way avoidance ($p < .001$); and two-way avoidance ($p < .001$). In particular, all experimental groups demonstrated dose response in either-way avoidance (Figure 2, $p < .001$, Table 4). RLA-S animals demonstrated dose response in one-way avoidance (Figure 3, $p < .01$, Table 4); and RHA-S ($p < .01$) and RLA-A ($p < .001$) animals demonstrated dose response in two-way avoidance (Figure 4, Table 4).

Further analyses showed that dose response was reflective of animals' reactions to the presence of the d-amphetamine, and was not dose related, because differences among three dosages of d-amphetamine were not significant (see Appendix A for complete derivation). Hence, results of further analyses (presented in Table 4) were based upon comparison of responses made under the effects of 1 mg/kg d-amphetamine to corresponding responses made under the effect of the placebo injection.

There was a three-way interaction (Table 4) among genetic lines X prenatal treatment X dose response in two-way avoidance (Figure 4, $p < .007$), as well as in one-way avoidance ($p < .03$, Figure 3, Table 4). In two-way avoidance, and to a lesser degree in one-way avoidance, there was a performance reversal between genetic lines depending upon the history of prenatal intervention, and the presence of exogenous d-amphetamine.

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In one-way avoidance (Figure 3) the RHA-A and RLA-S groups showed significant response facilitation under 1 mg/kg as compared to response rates in the placebo condition, and the RHA-S and RLA-A groups did not. The reverse was the case in two-way avoidance (Figure 4, Table 4), with RHA-S and RLA-A groups showing significantly increased avoidance under 1 mg/kg dose, as compared to respective amphetamine or saline prenatally treated groups.

Further analyses comparing the numbers of responses made under the effects of the placebo and 1 mg/kg doses (in one-and two-way avoidance) showed no statistically significant differences between genetic lines with the same history of prenatal pharmacological intervention, but a number of significant interactions were found (Table 4). In one-way avoidance a significant interaction was observed between genetic lines receiving saline prenatal treatment (Figure 3, $p < .001$), and in two-way avoidance (Figure 4) interactions were noted between genetic lines receiving amphetamine ($p < .003$) as well as saline ($p < .03$, see Table 4) prenatal treatments (refer to Appendix B for complete derivation).

As may be observed in either-way (Figure 2) and one-way (Figure 3) avoidance, there are marked discrepancies between performance on the last day of training (Day 4) and avoidance performance under the effect of the placebo. Comparison of the responses between last day of training

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and the placebo injection showed significant performance differences in the RLA-A animals in one-way (Figure 3) $\underline{F}(1,24) = 9.4$, $p < .006$; and either-way avoidance (Figure 2, $p < .002$). This could be indicative either of a placebo effect or carry-over effects of d-amphetamine, as approximately 75% of the animals had at least one d-amphetamine session before being tested with the placebo. Comparison of dosages by t-test, irrespective of order, revealed no significant differences between the mean number of avoidances made by animals first administered the placebo (M = 5.8), and following one (M = 5.6), two (M = 6.4), or three (M = 6.1) injections of d-amphetamine. This ruled out a possibility of carry-over effect. These findings are similar to those of Satinder (1977), in that animals of the RLA genetic line demonstrated significant placebo effects.

The mean number of mA administered to elicit UER were analysed according to genetic line and prenatal treatment (analysis carried out with n = 128). There were significant differences between genetic lines (RHA = .30 and RLA = .52 mA, $\underline{F}(1,113) = 61.3$, $p < .001$), but no significant differences between prenatal treatments.

Some of the literature on the effect of d-amphetamine prenatal treatment on subsequent learning lends support to the present findings. Studies have shown that rats prenatally treated with amphetamine made more conditioned avoidance responses than saline prenatally treated off-

spring (Martin, 1975; Nasello and Ramirez, 1978). Seliger (1973) has suggested that prenatal treatment with amphetamine enhances the tendency to respond, since the inability to withhold a response was noted in the impaired passive avoidance learning of animals prenatally treated with amphetamine. As indicated by results of the present study, increased avoidance responding or an enhanced tendency to respond may be influenced by arousal level, genetic background of the organism, and the complexity of the active avoidance response.

One drawback in the design of the present study involved the analysis of one-and two-way avoidance responses as components of the either-way task. The significant prenatal treatment X response complexity X genetic line interactions, under the effect of d-amphetamine, noted in one-and two-way avoidance (Figures 3 and 4, respectively) must be viewed within the context of avoidance performance as a composite of either-way responding. It is to be considered that avoidance performance was response complexity-specific, and not task specific per se. More definitive results may have been yielded had performance data been collected from responses made in either-way, one-way and two-way avoidance tasks, separately. However, it is to be recalled that originally sex differences were incorporated into the analysis; and for this reason it was thought that too few animals would be available for avoidance

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performance in each avoidance task, separately.

GENERAL DISCUSSION

Data on the effect of chronic prenatal d-amphetamine administration on adult learning behaviour have so far provided limited perspectives. In the present investigation the use of active avoidance behaviour with different levels of avoidance response complexity allowed for a finer analysis of the behavioural effects of prenatal exposure to d-amphetamine. These effects might not have been observed had only a single level of learning complexity been used. Present findings strongly suggest that prenatal d-amphetamine can affect avoidance learning in adulthood. At a more sophisticated level, the degree of learning complexity (higher, intermediate, or lower) which is affected by this intervention may also be examined. The data clearly indicated that prenatal d-amphetamine, (a) contributed to learning performance differences between genetic lines over four days of training, as noted previously, (b) was responsible for a marginally significant reduction in performance level of RLA-A animals over four days of training in the either-way task, (c) 1 mg/kg d-amphetamine significantly increased one-way avoidance of RHA-A animals, as compared to saline controls, (d) 1 mg/kg d-amphetamine significantly increased two-way avoidance of RLA-A animals, as compared to saline controls, (e) produced reversals in

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in performance as previously noted.

Satinder (1977, 1981 - in press) has proposed that the differences in avoidance behaviour of these lines of rats may have been derived from genetically related levels of arousal. The concept of physiological arousal supposes that the arousal level and efficiency of performance are related by an inverted U-shaped curve. In inverted-U arousal function, increases in arousal would be expected to facilitate performance up to an optimal level, for a given performance. Beyond that point further increases in arousal would result in decreased performance levels, proportionate to the increase in arousal levels:

> "Accordingly, the low-avoidance animals with a low level of arousal showed poor performance on a complex task (two-way task) in comparison with high-avoidance animals with a relatively higher baseline of arousal level. With the decrease in task complexity the low-avoidance animals with a lower baseline of arousal level showed improved performance (one-and either-way tasks). The high-avoidance animals with a high level of arousal showed a lower level of performance on simpler tasks (jump-up and passive tasks) compared with the low-arousal, low-avoidance animals." (Satinder, 1977, p 1335)

In addition to this proposal, present data provide a strong basis to speculate that prenatal treatment with d-amphetamine may have differentially altered the level of physiological arousal. This change in arousal level was reflected in avoidance behaviour during training as well as under the effect of d-amphetamine. Prenatal treatment with d-amphetamine may have increased the baseline arousal level of animals of the RHA genetic line,

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and decreased the baseline arousal level of animals of the RLA genetic line. This could explain the differences in performance during training in two-way avoidance between RHA-A and RHA-S animals: With an increase in baseline arousal, effective avoidance responding in a complex mode (two-way avoidance) was enhanced (as seen in performance of RHA-A animals), thereby reducing avoidance responding in a less complex mode (one-way avoidance). Under the effects of d-amphetamine RHA-A animals showed poorer performance at a higher level of response complexity (two-way avoidance) and improved performance at an intermediate level of response complexity (one-way avoidance). This could have been because the stimulant effects of d-amphetamine injection had raised the level of physiological arousal in these animals beyond the optimal performance level for effective avoidance responding in a complex mode (two-way avoidance), but not beyond the optimal performance level for effective avoidance responding in a less complex mode (one-way avoidance).

An explanation for the decreased two-way performance of RLA-A animals observed during training may be provided by the suggestion that lowered baseline arousal levels in RLA animals were attributable to prenatal treatment with d-amphetamine. Under the effect of d-amphetamine RLA-A animals did not significantly increase performance at an intermediate level of response complexity as did RLA-S animals, but did significantly increase performance at the

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higher level of response complexity (two-way avoidance). This could have been because the stimulant effects of d-amphetamine injection had raised the level of physiological arousal to a more optimal performance level in these animals (i.e., RLA-A animals), thereby facilitating avoidance responding in a complex mode. Avoidance responding in a less complex mode was reduced, suggesting that response trends reflecting directionality were more clearly indicated by RLA-A animals in two-way avoidance than by RLA-S animals. While it has been previously noted (Satinder, 1977; Satinder and Petryshyn, 1974) that the low-avoidance line lacks directionality, present findings suggest that depending upon prenatal treatment, a relationship may indeed exist between arousal and directionality involving animals of the RLA genetic line.

The suggestion that prenatal amphetamine alters baseline levels of physiological arousal is not inconsistent with the literature: Data on open-field behaviour (Hitzemann, et al., 1976; Middaugh et al., 1974; Nasello and Ramirez, 1978; Seliger, 1973), and activity wheel performance (Martin, et al., 1976), have shown that regardless of differing doses, period of treatment, or even species, amphetamine prenatally treated adult offspring demonstrate higher activity levels than saline prenatally treated offspring. The relation between arousal and activity has previously been proposed in the two Roman lines of rats.

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In a recent review of the Roman lines Satinder (1981 - in press) has demonstrated that activity can indeed be linked to arousal. He derived support from Sartory and Eysenck (1976, p 167) who state that "the Roman strains have to be considered as a low and high arousal strains... Animals characterized by high levels of arousal exhibit a higher degree of general activity."

As well, the suggestion that amphetamine prenatal treatment alters baseline levels of physiological arousal could explain why prenatally treated rats made more errors than saline controls in a Lashley III maze (Nasello and Ramirez, 1978). (Increased activity levels, reflected by greater numbers of errors, could have been incompatible with that particular maze learning). The suggestion could also explain why decreased passive avoidance performance (Seliger, 1973) has been noted in rats receiving prenatal amphetamine. Seliger (1973) has suggested that the inability to withhold a response, thereby increasing time to learning, may be a function of a higher activity level. However, the inability to withhold a response could also be interpreted as a function of increased arousal, reflected in higher activity. The additional stress of footshock, which has previously been shown to affect arousability (Satinder, 1977, citing Gray, 1964), may have acted to further increase arousal. The resulting arousal level was incompatible with successful passive avoidance learning.

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In contrast to Seliger's findings (1973), the effect of amphetamine prenatal treatment may not be just to enhance the tendency to respond. There may be higher activity levels as suggested by Seliger (1973) associated with this prenatal intervention, but other studies have more recently demonstrated that the results of amphetamine prenatal treatment may have a more specific physiological effect. To account for some of these effects Nasello and Ramirez (1978) have proposed that the "excitability levels" of prenatal amphetamine offspring are higher. Perhaps more simply restated, amphetamine prenatal treatment may serve to alter baseline levels of physiological arousal.

In conclusion, while it is argued that amphetamine prenatal treatment is associated with altered levels of arousal, the effects of such change on learning performance may be task-specific. It has been reported that performance associated with observed higher activity levels has not been increased in some learning tasks (eg., the Lashley III maze, and passive avoidance tasks). The response required - inhibition of a particular behaviour - is incompatible with the suggested higher level of arousal. On the other hand, performance on learning tasks requiring response initiation (active avoidance tasks) may be improved, for the response required is compatible with, and may be facilitated by, the suggested higher level of arousal (Martin, 1975; Nasello and Ramirez, 1978; and present

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findings). The observation of discrete changes in avoidance behaviours of varying complexity following prenatal amphetamine intervention, under the effects of d-amphetamine as well as during training, would indicate the degree of this facilitation may be affected by genetic selection and the complexity of the response.

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Appendix A

Analysis of Variance Summary of Interaction Effects for Placebo and Drug Levels

0 x 1 x 2 x 4 mg/kg d-amphetamine

Source	DF	SS	MS	F
W12	3	0.88	0.29	0.0683
W12B1	3	6.17	2.06	0.4773
W12B2	3	0.54	0.18	0.0420
W12B12	3	65.84	21.95	5.0907**
EW12B12	288	1241.68	4.31	

0 mg/kg x 1 mg/kg d-amphetamine

Source	DF	SS	MS	F
W12	1	0.00	0.00	0.0007
W12B1	1	3.06	3.06	0.9151
W12B2	1	0.20	0.20	0.0605
W12B12	1	63.20	63.20	18.8852***
EW12B12	96	321.28	3.35	

1 x 2 x 4 mg/kg d-amphetamine

Source	DF	SS	MS	F
W12	2	1.54	0.77	0.1559
W12B1	2	3.37	1.68	0.3403
W12B2	2	0.67	0.34	0.0677
W12B12	2	29.79	14.90	3.0084
EW12B12	192	950.63	4.95	

** $p < .01$

*** $p < .001$

W1 = task complexity
W2 = dosage levels
B1 = genetic lines
B2 = prenatal treatment

Appendix B

Analysis of Variance Summary of Interaction Effects According to Genetic Line, Under 0 mg/kg X 1 mg/kg d-Amphetamine

One-way avoidance, saline prenatal treatment

Source	DF	SS	MS	F
W1	1	31.36	31.36	10.1571**
W1B1	1	38.44	38.44	12.4502***
EW1B1	48	148.20	3.09	

Two-way avoidance, amphetamine prenatal treatment

Source	DF	SS	MS	F
W1	1	24.01	24.01	11.2547**
W1B1	1	22.09	22.09	10.3547**
EW1B1	48	102.40	2.13	

Two-way avoidance, saline prenatal treatment

Source	DF	SS	MS	F
W1	1	26.01	26.01	11.3251**
W1B1	1	12.25	12.25	5.3338*
EW1B1	48	110.24	2.30	

* $p < .05$
** $p < .01$
*** $p < .001$

W1 = dosage levels
B1 = genetic lines